

Enhanced Crude Oil Biodegradation in a Two-liquid Phase Partitioning Bioreactor

Z. Z. Ismail^{a,*} and I. A. Abdulrazzak^b

doi: 10.15255/CABEQ.2014.2080

^aDepartment of Environmental Engineering, University of Baghdad, Baghdad, Iraq

Original scientific paper

Received: July 16, 2014

^bMinistry of Environment, Baghdad, Iraq

Accepted: September 3, 2015

The biodegradation of crude oil at relatively high concentrations in an aquatic environment is constrained by the inherent toxicity of crude oil. In this study, a new application of an aqueous- organic two-liquid phase partitioning bioreactor (TLPPB) was developed to degrade high concentrations of crude oil up to 5000 mg L⁻¹. Silicon oil was selected as the sequestering organic phase to control the delivery of crude oil in aqueous phase by absorbing, and subsequently releasing the crude oil to the microorganisms. The efficiency of the silicon oil- based TLPPB has been compared to the conventional monophasic bioreactor. Regardless of the crude oil concentrations and the type of aqueous phase, complete removal of crude oil up to 100 % was achieved in the two-liquid phase partitioning bioreactor (TLPPB) compared to 75–80 % removal efficiency of crude oil in the monophasic conventional bioreactor. In addition, the performance of TLPPB for crude oil removal was evaluated in terms of the salinity effect using distilled, river, and marine water samples. High salinity of aqueous phase proved to slightly inhibit the microorganisms-bioactivity and hinder the rate of crude oil degradation in the TLPPB. This study indicates the potential of TLPPB for enhancing the transport and biodegradation of crude oil in aqueous solutions.

Key words:

crude oil, biodegradation, biphasic bioreactor, salinity, silicon oil

Introduction

One of the most dramatic environmental problems is the crude oil spill into natural aquatic systems and the subsequent environmental risks represented by endangering parts of the aquatic and ecosystem due to oil seepage from damaged pipelines into water supplies. Accidental leakages during hydrocarbon fuels transportation and other activities are inevitable, making these hydrocarbons the most common global environmental pollutants. The spill of oil into water is raising concerns of human health as well as ecological damage¹. Biological treatment is considered a cost-effective environmentally friendly technology that is often used to treat oil spills in soils, groundwater, and surface water^{2,3}. Due to the obvious benefit of the actual removal of oil from the environment and the fact that it can be cost effective compared to other physical and chemical treatment technologies, biodegradation has been, and currently remains, the subject of considerable research⁴. In the biotreatment of any organic compound, the most significant challenge is sub-

strate delivery. That is, the addition of substrate at high concentrations will inhibit or even kill the organisms, whereas substrate addition at too low concentrations will cause the cells to starve and result in a sub-optimal process performance, so that precise and controlled delivery of these materials is exceedingly important⁵. The two-phase partitioning bioreactor concept (TPPB) appears to have great potential in enhancing the productivity of many bioprocesses. The proper selection of an organic solvent is the key to the successful application of this approach in industrial practices. The controlled substrate delivery from the organic to the aqueous phase opens a new area of application of this strategy to biodegradation of pollutants. In such a two-phase aqueous–organic system, the substrate is solubilized in the immiscible organic phase and allowed to transfer into the organic phase. The microorganisms degrade or transform the substrate at the aqueous/organic interface and/or in the aqueous phase. Thus, the substrate concentration in the biotic phase can be maintained below the inhibitory level. The partition process itself is controlled to some extent by the metabolic activity of microorganisms. The system is well suited for biodegradation of hazardous pollutants^{6,7}. The idea of a two-

*Corresponding author: e-mail: zismail9@gmail.com; zismail3@gatech.edu, tel: +9647709023240

phase partitioning bioreactor was first proposed by Collins and Daugulis⁸ for the biodegradation of xenobiotic pollutants that originally exists in the organic solvent. Tomei *et al.*⁷ compared the performance of a TPPB, relative to single phase operation, in which a small volume (5 %, v/v) of the beads polymer Hytel 8206 was used to treat aqueous mixtures of 2,4-dimethylphenol and 4-nitrophenol. Zuniga *et al.*⁹ studied the biodegradation of methane, and the accumulation of poly-hydroxybutyrate using a methanotrophic consortium and an isolated strain in a TPPB. Karimi *et al.*¹⁰ designed a lab-scale bioreactor for treatment of waste gas containing benzene, toluene and xylene (BTX). Ramos *et al.*¹¹ proposed a two phase partitioning bioreactor to carry out the degradation of the poorly soluble compound anthracene by laccase from *Trametes versicolor*. The organic phase consisted of silicone oil saturated with anthracene. Juang *et al.*¹² used a two-phase partitioning bioreactor equipped with an agitator to remove phenol from an organic phase that was dispersed in an aqueous mineral salt medium containing *Pseudomonas putida* BCRC 14365. Kerosene was selected as the organic solvent. Munoz *et al.*¹³ investigated an innovative operation mode in a two-liquid phase partitioning bioreactor (TLPPB) for the treatment of volatile organic compounds (VOC). The TLPPB was implemented in a 2.5 L stirred tank reactor using 10 % (v/v) of silicone oil as the organic phase and hexane as model VOC. A removal efficiency of 80 % was recorded for 26 days. However, none of the previously reported studies involved the crude oil biodegradation in a two-liquid phase partitioning bioreactor (TLPPB). Ismail and Abdulrazzak¹⁴ studied the anaerobic biodegradation of crude oil in mineral salt media (MSM) using small bench scale-TLPPB. The results revealed that complete removal of crude oil was achieved in biphasic bioreactor after 3 weeks compared to 73–82 % in a monophasic bioreactor for the same period.

The main objective of this study was to evaluate for the first time the biodegradation of crude oil in actual river and marine water samples using TLPPB inoculated with mixed culture at anaerobic conditions. The effects of aqueous phase salinity, substrate initial concentration, and agitation rate were evaluated.

Materials and methods

Aqueous and non-aqueous solutions

Two types of actual aqueous solutions were examined in this study which were river water (RW) samples freshly collected from the Tigris River (Iraq), and marine water (MW) samples collected

Table 1 – Properties of water samples

Constituent	Average concentration, mg L ⁻¹		
	distilled water (DW)	river water (RW)	marine water (MW)
TSS	–	23	117
TDS	–	567	46000
BOD ₅	–	1.3	12
COD	–	Nil	21
Oil content	–	Nil	Nil
NH ₄ ⁺	–	0.36	1.25
NO ₃ ⁻	–	4.7	6.9
PO ₄ ³⁻	–	0.38	0.46
Cl ⁻	–	138	24400
SO ₄ ²⁻	–	243	4212
pH	7.0	8.0	7.7

from the Arabian Gulf. For comparison purpose, a set of experiments was conducted with distilled water (DW) to demonstrate the effect of total dissolved solids (TDS) concentration on the biotreatment process. The properties of the water samples are given in Table 1. The aqueous samples were synthetically contaminated with crude oil obtained from the Midland Refineries Company-Al-Dora Refinery (Iraq). The properties of the crude oil samples are given in Table 2. Silicone oil (polydimethylsiloxane) was selected in this study as the organic phase solvent, supplied by Gainland Chemical Company (GCC), UK.

Table 2 – Properties of the Al-Basrah crude oil

Constituent	Value	Unit
Density	0.8745	mg mL ⁻¹
Viscosity	0.055	N · s m ⁻²
API gravity	30.3	–
Pour point	–30	°C
Water content	nil	%wt
Salt content	10	Ptb*
Sulphur	3.1	%wt
CCR	5.9	%wt
Wax	1.2	%wt
Asphaltene	2.6	%wt
ASTM distillation cracking point	339	°C

*pound per thousand barrels

Microorganisms and growth media

The mixed culture was freshly collected from the bottom of an aeration tank at a local sewage treatment plant (Baghdad, Iraq). The stock cultures were stored at 4 °C. The Mineral Salt Media (MSM) used for maintenance and culturing of the microorganisms was prepared at pH 6.8. The composition of MSM in (mg L⁻¹) was, (NH₄)₂SO₄ (100), KH₂PO₄ (350), K₂HPO₄ (775), MgSO₄ · 7H₂O (100), CaCl₂ (40), FeSO₄ · 7H₂O (1.0), MnSO₄ · H₂O (1.0), NaMoO₄ (0.21), NaCl (5000), meat papain peptone (15000), and Tryptone (15000). Prior to use, the MSM were sterilized in an autoclave at 121 °C for 30 minutes. The pH was fully controlled by daily monitoring and the final pH was 7.

Analytical procedure

Analysis of oil content was carried out using the oil content analyzer (Model: HORIBA OCMA-350) based on infrared analysis. It included a single-beam, fixed wavelength, non-dispersive infrared filter-based spectrophotometer. Infrared radiation from a tungsten lamp was transmitted through a cylindrical, quartz cuvette containing a sample extract. The radiation that passed through the extract entered a detector containing a filter that isolated analytical wavelengths in 3400 to 3500 nanometer range. Chemical oxygen demand (COD) concentrations in aqueous samples were measured using the COD analyzer (Model: Lovibond, RD 125). Volatile suspended solids (VSS) concentrations were measured according to *Standard Methods*¹⁵. Measurements of produced biogas were carried out using gas chromatography (Model: PACKARD 438A). Biomass growth was observed using scanning electron microscopy (SEM) (model: VEGA /easy probe). An additional approach known as standard plate count was used in this study to quantitatively measure the bacteria growth.

Microorganisms' cultivation

Freshly collected activated sludge samples were centrifuged at 3000 rpm for 10 minutes to separate the active biomass from liquid phase. The separated suspension of cultures were activated and enriched at 30±2 °C in 250 mL nutrient media (MSM), into which 20 mg L⁻¹ of crude oil was added to 10 mL of mixed culture suspension in a 500 mL-Erlenmeyer flask to be adapted for 24 h. Prior to starting the adaptation process, the flasks were flushed with nitrogen to achieve an anaerobic environment, and then agitated by an orbital shaker at 100 rpm to enrich the culture seeds. The growing mixed cells were collected after centrifugation of the enriched suspension at 3000 rpm for 10 minutes. The collected activated cells were re-suspend-

ed and re-inoculated into fresh culture media in 500 mL-Erlenmeyer flasks with the addition of 80 mg L⁻¹ crude oil. After inoculation, the flasks were flushed with N₂, capped and placed in an orbital shaker controlled at 100 rpm and 28±2 °C. These cultivation cycles were sequentially repeated for 20 cycles with increased initial concentrations of crude oil up to 5000 mg L⁻¹. For each cultivation cycle, the initial and final concentrations of cells were determined.

Biodegradation of crude oil in TLPPB

The anaerobic biodegradation process of crude oil in aqueous solution was extended to a Lab-scale TLPPB. A 12-liter glass vessel TLPPB with a thermostatically controlled water jacket to maintain the temperature at 30±2 °C was used in this study. The TLPPB was provided with a two level 4-bladed turbine for agitation, and it was covered with a stainless steel cover containing 4 ports for: (1) feed inlet, (2) nitrogen flushing, (3) acid/base addition for pH control, and (4) biogas discharge (Fig. 1). After the activated cells were inoculated in the culture medium, it was placed in the TLPPB; in the meantime, the aqueous solutions containing crude oil were poured into the vessel, followed by the addition of silicon oil, and then the vessel was flushed with nitrogen to maintain anaerobic environment. Samples for analysis were taken from the aqueous and organic phases at constant time intervals. Each experiment was at least duplicated under similar conditions.

Results and discussion

Biodegradation of crude oil in TLPPB shake-flasks

Effect of crude oil initial concentration and salinity

The results revealed that when initial concentration of crude oil was 1000 mg L⁻¹, complete reduction of COD and oil content were observed after 21, 30, and 41 days in distilled, river, and marine water samples, respectively. At higher concentration of crude oil up to 5000 mg L⁻¹, slightly extended time was required to achieve complete removal of crude oil, i.e., 23, 37, and 49 days in distilled, river, and marine water samples, respectively (Fig. 2). On the other hand, salinity of the aqueous solution is an essential parameter in biotreatment processes as it could affect the biomass growth and the subsequent biodegradation rate. Limitations of mass transfer and toxic effects were expected at high salinity concentration. However, in spite of the high TDS concentration in marine water samples, complete removal of crude oil up to 100 % was observed but at lower degradation rate. This variation in the durations for complete degradation of crude oil in the

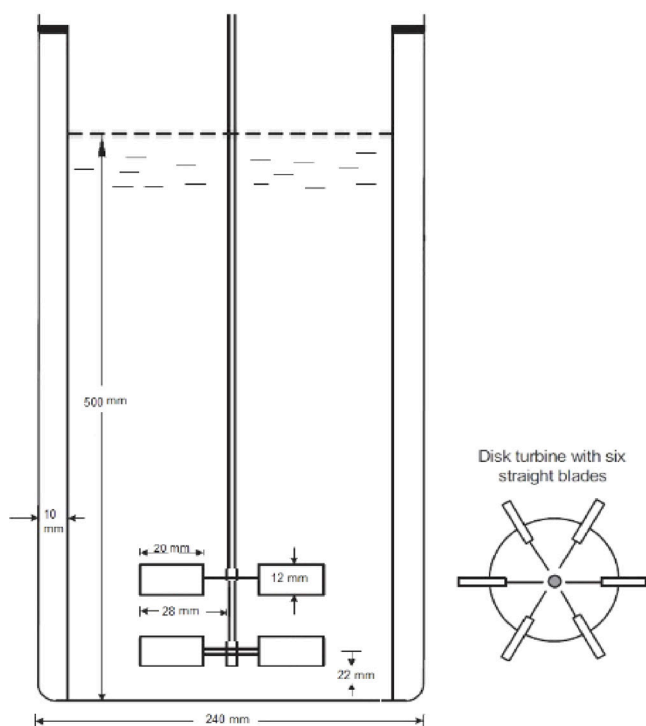


Fig. 1 – Lab-scale TLPPB used for crude oil removal

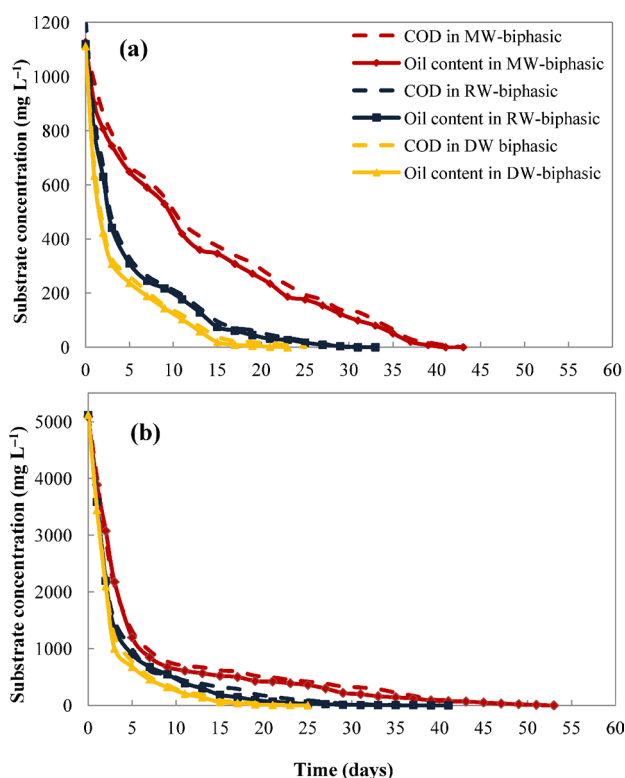


Fig. 2 – Effect of salinity and initial concentration

three types of aqueous solutions could be attributed to the effect of salinity because high TDS concentration is a stressful agent for most of organisms which may hinder their activity and the subsequent rate of the oil biodegradation process.

These findings are in a good agreement with the previously reported investigations. Minai-Tehrani *et al.*¹⁶ observed 41 % crude oil degradation in soil samples with no NaCl added, while only 12 % was obtained in samples from the same soil subject to 50 g L⁻¹ NaCl after 120 days. The negative impact of increasing salinity on hydrocarbons biodegradation is also observed in environments where halotolerant and/or slightly halophilic microorganisms tend to be dominant, as the case of mangroves^{17,18}. Even in typical hyper-saline environments, a negative impact on hydrocarbon biodegradation is induced by increasing salinity. Mille *et al.*¹⁹ reported that the amount of oil degraded decreased with increasing salt concentrations.

Effect of agitation rate

A set of experiment were carried out using river water as an aqueous phase to study the effect of agitation rate on oil removal in TLPPB. Fig. 3 illustrates the effect of agitation rate on the crude oil removal. It is well observed that at 250 rpm agitation rate, the crude oil removal rate was faster than at 120 rpm as more time was required to achieve equilibrium. These results could be attributed to the fact that higher mass transfer can be obtained by increasing the mixing rate. These findings are in a good agreement with the previously reported results by Juang *et al.*²⁰ for the removal of phenol in (TPPB).

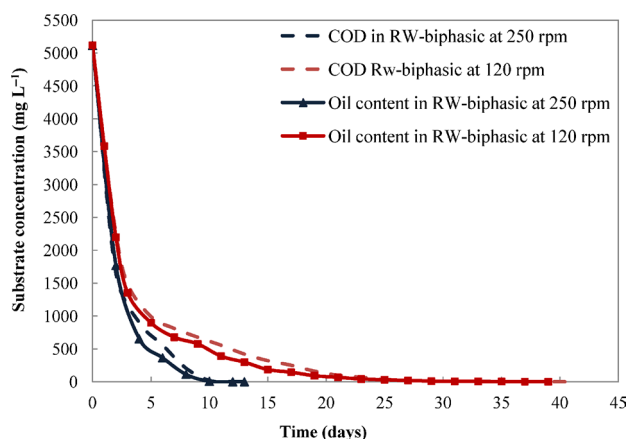


Fig. 3 – Effect of agitation rate on crude oil removal

However, the main objective of mixing is to maximize the process rate. It would be reasonable to believe, nonetheless, that increasing the agitating rate could increase the total surface contact and consequently the speed of reaction. A minimum requirement for the process is that the concentration is well distributed throughout the mixing volume²¹.

Comparison of crude oil removal between monophasic and biphasic bioreactors

To quantify the action of the organic phase on the degradation rate, a set of experiments were conducted without using silicon oil as the organic extractive phase. Results given in Table 3 indicate that the microbial activity for degradation of crude oil was greatly enhanced in the biphasic system. In case of monophasic bioreactor, microorganisms were in direct contact with high concentration of crude oil up to 5000 mg L⁻¹ and the microbial growth seemed to be limited or hindered. Comparative biodegradation of crude oil in the biphasic and monophasic system emphasize the role of the organic extractive phase. The silicon oil seems to act as a sponge that absorbs the crude oil droplets from the aqueous phase, and then by continuous shaking

Table 3 – Removal efficiencies of crude oil at 5000 mg L⁻¹ initial concentration

Reactor type	Max. removal, %	Duration of biodegradation, days
DW-monophasic	80 %	21
RW-monophasic	78 %	31
MW-monophasic	75 %	49
DW-biphasic	100 %	21
RW-biphasic	100 %	30
MW-biphasic	100 %	49

and agitation the organic phase will gradually release the substrate to the microorganism in the aqueous media.

Biodegradation of crude oil in lab-scale TLPPB

Experimental work was extended to study the performance of the lab-scale TLPPB system alternatively using river and marine water samples-loaded with crude oil at initial concentration of 5000 mg L⁻¹. The results revealed that complete removal of crude oil up to 100 % in river and marine water samples were achieved after 10 and 20 days, respectively (Fig. 4). In these experiments, no by-products were observed since complete biodegradation of oil was achieved. However, measurements of the released biogas indicated that CH₄ and CO₂ were the end-products of the oil anaerobic biodegradation in TLPPB.

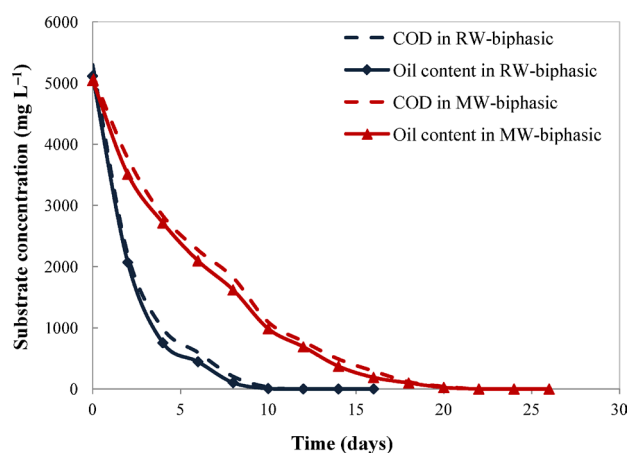


Fig. 4 – Profiles of substrate removal in river (RW) and marine (MW) water samples in lab-scale TLPPB

The results of this study indicated complete removals of oil, which is in a good agreement with the previously published investigations. Yeom *et al.*²² reported that the removal efficiency of benzene was more than 99 % within 24 h in a Lab-scale TPPB. MacLeod & Daugulis²³ reported that phenanthrene and pyrene were completely degraded within 4 days in a Lab-scale TPPB.

In order to examine the validity and reliability of the suggested approach, it was necessary to test the aqueous samples actually contaminated with crude oil resulted from an accidental spill. These samples were collected from the Tigris River (Baiji, Iraq). Table 4 presents the quality of the actually contaminated aqueous samples. A set of experiments was conducted using the actual samples of crude oil-contaminated river water alternatively in Lab-scale monophasic and biphasic bioreactor (TLPPB). The results revealed complete removal of crude oil after 6 days in TLPPB at an average

Table 4 – *Quality of the river-water samples actually contaminated with crude oil*

Constituent	Average concentration, mg L ⁻¹
TSS	41
TDS	314
BOD ₅	0.90
COD	3100
Oil content	3070
NH ₄ ⁺	0.21
NO ₃ ⁻	3.80
PO ₄ ³⁻	0.16
Cl ⁻	64.6
SO ₄ ²⁻	97
pH	7.80

initial concentration of 3000 mg L⁻¹, while the removal efficiency of crude oil in the monophasic reactor did not exceed 85 % and remained constant (Fig. 5).

Biomass granulation

Upon achieving equilibrium in the TLPPB, the biomass granules formation was clearly visible. This granulated biomass had a typical brownish-dark gray color and 1–2 mm size of granules. The Scanning Electron Microscopy (SEM) images

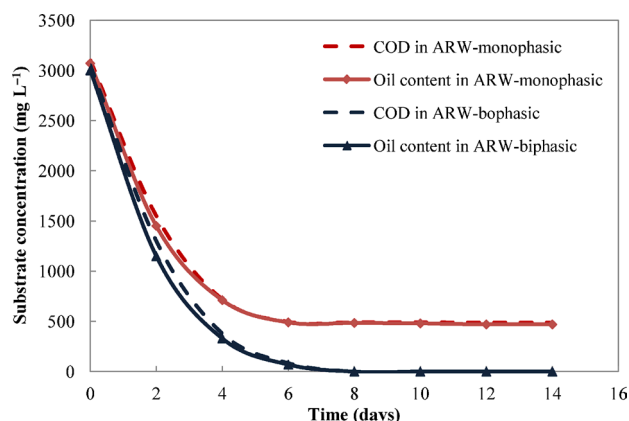


Fig. 5 – *Profiles of substrate removal in actually contaminated river-water (ARW) samples in monophasic and biphasic bioreactors*

of these bio-granules (Fig. 6) revealed a porous and spongy structure with some cracks on the surface of the layers. A similar observation was reported by Amro²⁴ for biomass growth on oil contaminated-soil samples using the SEM technique.

Conclusion

This study confirmed the potential of the biphasic bioreactor (TLPPB) for the anaerobic biodegradation of crude oil for concentrations up to 5000 mg L⁻¹ in aqueous phase using silicon oil as the extractive organic phase solvent. The TLPPB significantly enhanced the biological degradation

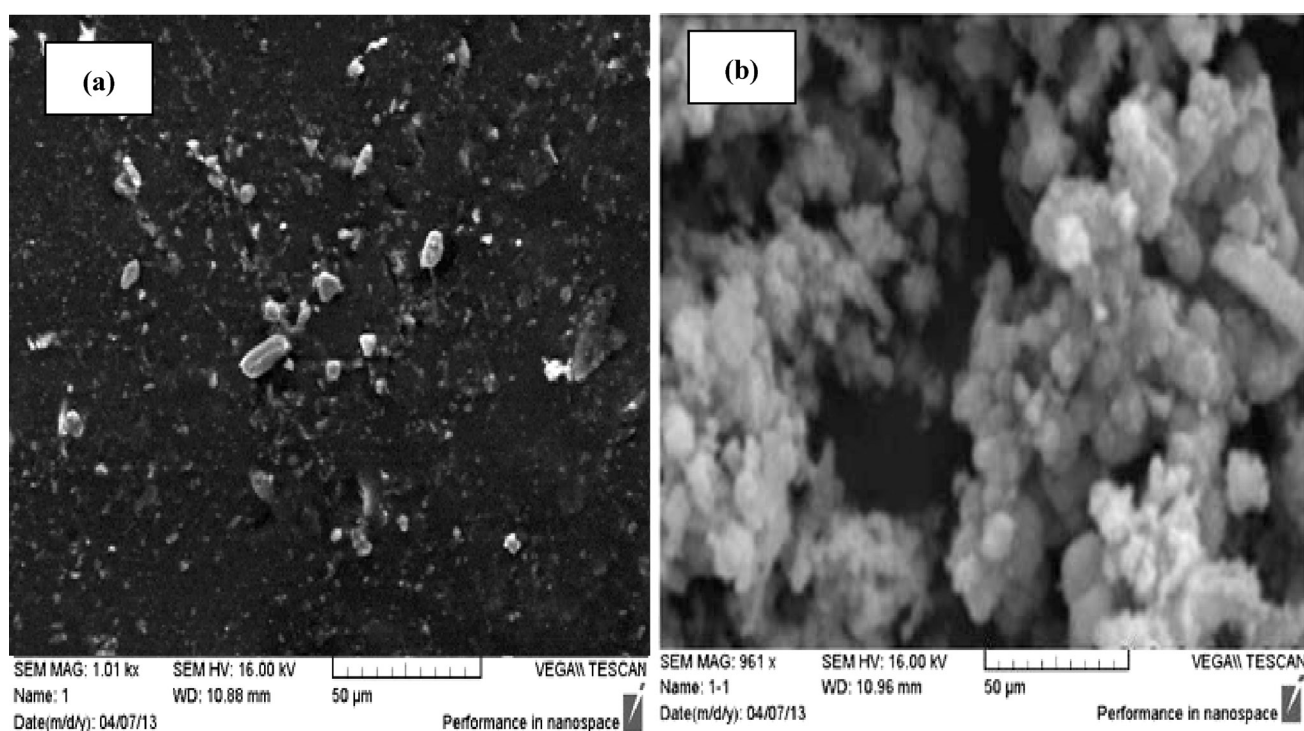


Fig. 6 – *SEM images for mixed culture (a) before growth and (b) after growth in TLPPB*

rate of crude oil and reduced the inhibitory and toxicity effect of crude oil on mixed culture. Under the conditions studied, the crude oil was completely removed and biodegraded in the lab-scale TLPPB within 10 and 20 days at 5000 ppm crude oil in river and marine water samples, respectively. The salinity of the aqueous phase affected and hindered the biomass activity causing longer time necessary for the complete removal of crude oil.

ACKNOWLEDGEMENT

Ministry of Environment, Iraq is greatly acknowledged for their financial support. University of Technology is also acknowledged for SEM support.

References

1. Darracq, G., Annabelle, C., Catherine, C., Diane, T., Abdeltif, A., Eric, D., Yves, A., Pierre, L. C., Optimization of the volume fraction of the NAPL, silicone oil, and biodegradation kinetics of toluene and DMDS in TPPB, *Int. Biodeterior. Biodegrad.* **71** (2012) 9.
2. Prince, R., McMillen, S., Summary of PERF Bioremediation Projects. Presentation at DOE/PERF Bioremediation Workshop. Houston, Texas (2002).
3. Hernandez, M., Quijano, G., Thalasso, F., Daugulis, A. J., Villaverde, S., Munoz, R. A., A comparative study of solid and liquid non-aqueous phases for the biodegradation of hexane in two phase partitioning bioreactors, *Biotechnol. Bioeng.* **106** (2011) 731.
doi: <http://dx.doi.org/10.1002/bit.22748>
4. Eibes, G., McCann, C., Pedezert, A., Moreira, M. T., Feijoo, G., Lema, J. M., Study of mass transfer and biocatalyst stability for the enzymatic degradation of anthracene in a two-phase partitioning bioreactor, *Biochem. Eng. J.* **51** (2010) 79.
doi: <http://dx.doi.org/10.1016/j.bej.2010.05.006>
5. Ramos, A. A., Eibes, G., Moreira, M. T., Feijoo, G., Lema, J. M., Mass Transfer Enhancement by the Addition of Surfactant in a Two Phase Partitioning Bioreactor for the Degradation of Anthracene by Laccase, *J. Chem. Eng. Trans.* **27** (2012) 187.
6. Janusz, J., Malinowski, A., Two-phase partitioning bioreactors in fermentation technology, *Biotechnol. Adv.* **19** (2001) 525.
doi: [http://dx.doi.org/10.1016/S0734-9750\(01\)00080-5](http://dx.doi.org/10.1016/S0734-9750(01)00080-5)
7. Tomei, C. M., Sara, R., Domenica, M. A., Cristina, A. M., Daugulis, A. J., Treatment of substituted phenol mixtures in single phase and two-phase solid–liquid partitioning bioreactors, *J. Hazard. Mater.* **19** (2011) 190.
doi: <http://dx.doi.org/10.1016/j.jhazmat.2011.04.061>
8. Collins, L. D., Daugulis, A. J., BTX degradation part I: solvent selection and toluene degradation in a two-phase partitioning bioreactor, *Appl. Microbiol. Biotechnol.* **52** (1999) 354.
doi: <http://dx.doi.org/10.1007/s002530051531>
9. Zuniga, C., Morales, M., Le, B. S., Revah, S., Production of poly-hydroxybutyrate (PHB) by *Methylobacterium organophilum* isolated from a methanotrophic consortium in a two-phase partition bioreactor, *J. Hazard. Mater.* **190** (2011) 876.
doi: <http://dx.doi.org/10.1016/j.jhazmat.2011.04.011>
10. Karimi, A., Golbabaie, F., Neghab, M., Mehrnia, M. R., Mohammad, K., Pourmand, M. R., Nikpey, K., Investigation of Oxygen Transfer in a Two-phase Partition Stirred Tank Bioreactor in the Presence of Silicone Oil, *Chem. Biochem. Eng. Q.* **25** (2011) 160.
11. Ramos, A., Eibes, G., Moreira, M. T., Feijoo, G., Lema, J. M., Surfactant-assisted two phase partitioning bioreactors for laccase-catalyzed degradation of anthracene, *Process Biochem.* **47** (2012) 1115.
doi: <http://dx.doi.org/10.1016/j.procbio.2012.04.002>
12. Juang, R. S., Kao, H. C., Zhao, Z., A simplified dynamic model for the removal of toxic organics in a two-phase partitioning bioreactor, *Sep. Purif. Technol.* **90** (2012) 213.
doi: <http://dx.doi.org/10.1016/j.seppur.2012.02.038>
13. Munoz, R., Eleonora, I. H., Ganb, H., Hernandez, M., Quijano, G., Hexane biodegradation in two liquid phase bioreactors: High performance operation based on the use of hydrophobic biomass, *Biochem. Eng. J.* **70** (2013) 9.
doi: <http://dx.doi.org/10.1016/j.bej.2012.09.009>
14. Ismail, Z. Z., Abdulrazzak, I. A., Two-Phase Partitioning Bioreactor for the Treatment of Crude Oil-Contaminated Aqueous Solution, *Engineering* **20** (2014) 129.
15. APHA, Standard methods for examination of water and wastewater, American Public Health Association, Washington, DC (1998).
16. Minai, T. D., Herfatmanesh, A., Azari, D. F., Minuoi, S., Effect of salinity on biodegradation of aliphatic fractions of crude oil in soil, Pakistan. *J. Biol. Sci.* **9** (2006) 1531.
doi: <http://dx.doi.org/10.3923/pjbs.2006.1531.1535>
17. Diaz, M. P., Boyd, K. G., Grigson, S. G. W., Burgess, J. G., Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibers, *Biotechnol. Bioeng.* **7** (2002) 145.
doi: <http://dx.doi.org/10.1002/bit.10318>
18. Santos, H. F., Carmo, F. L., Paes, J. E. S., Rosado, A. S., Peixoto, R. S., Bioremediation of Mangroves Impacted by Petroleum, *Water Air Soil Pollut.* **216** (2011) 329.
doi: <http://dx.doi.org/10.1007/s11270-010-0536-4>
19. Mille, G., Almallah, M., Bianchi, M., VanWambeke, F., Bertrand, J. C., Effect of salinity on petroleum biodegradation, *J. Anal. Chem.* **339** (1991) 788.
doi: <http://dx.doi.org/10.1007/BF00321746>
20. Juang, R. S., Kao, H. C., Tseng, K. J., Kinetics of phenol removal from saline solutions by solvent extraction coupled with degradation in a two-phase partitioning bioreactor, *Sep. Purif. Technol.* **71** (2010) 285.
doi: <http://dx.doi.org/10.1016/j.seppur.2009.12.008>
21. Rissler, S., Mixing and its role in Biological Treatment-proper process design of anoxic zones with reference to Mixing. Article from Scientific Impeller (1991).
22. Yeom, S. H., Daugulis, A. J., Development of a novel bioreactor system for the treatment of gaseous benzene, *Biotechnol. Bioeng.* **72** (2001) 156.
doi: [http://dx.doi.org/10.1002/1097-0290\(20000120\)72:2<156::AID-BIT4>3.0.CO;2-V](http://dx.doi.org/10.1002/1097-0290(20000120)72:2<156::AID-BIT4>3.0.CO;2-V)
23. Macleod, C. T., Daugulis, A. J., Biodegradation of polycyclic aromatic hydrocarbons in a two-phase partitioning bioreactor in the presence of a bioavailable solvent, *Appl. Microbiol. Biotechnol.* **62** (2003) 291.
doi: <http://dx.doi.org/10.1007/s00253-003-1297-4>
24. Amro, M. M., Treatment techniques of oil-contaminated soil and water aquifers. Proceeding of International Conference on Water Resources & Arid Environment, Petroleum Engineering Department, King Saud University, Saudi Arabia (2004).